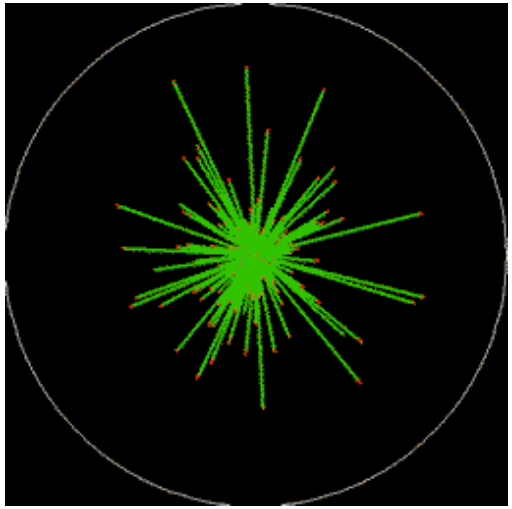
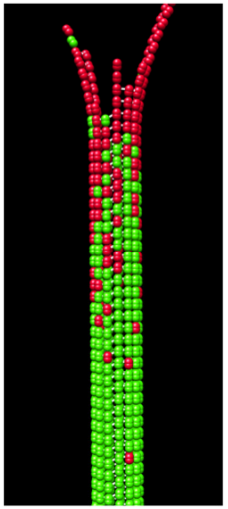
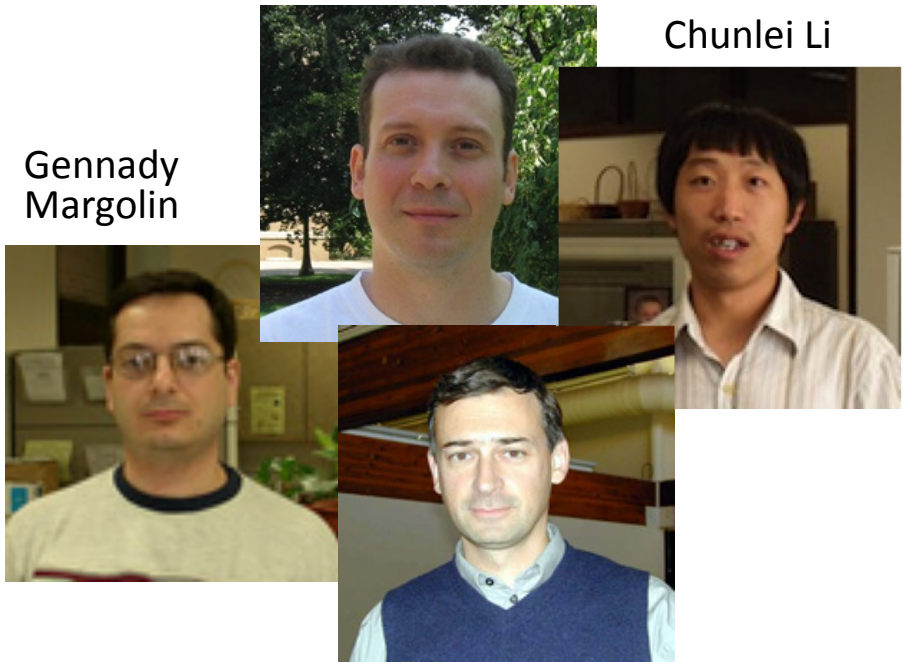
Biological Insights from Computational Modeling of Microtubule Dynamics







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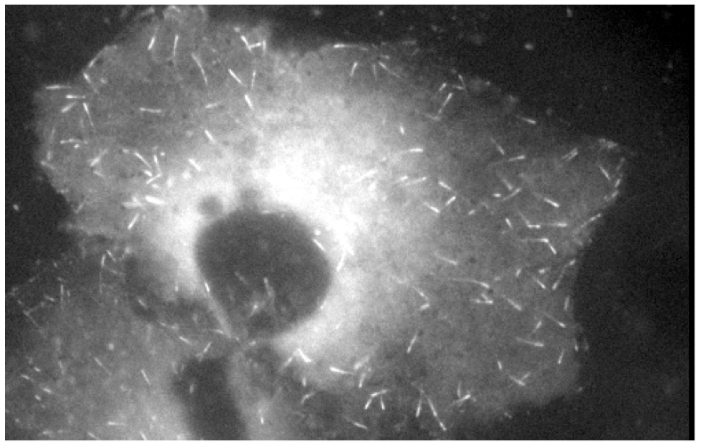
Long-term interest: MT cytoskeleton in cell function



GFP-CLIP-170
In COS-7 cells

MT binding proteins

- effect on MT dynamics
- mechanism of effect on MT dynamics

Problem:

- How does one make **quantitative predictions** about effect of a MT binding protein on MT dynamics?
- How can you make *any* quantitative predictions about MT dynamics?

Basic Questions:

- What happens if a given amount of tubulin is polymerized?
  How much polymer? MT lengths? Transition frequencies?
- How are answers influenced by:
  # nuclei? spatial constraints of a cell? MAPs?
- Mechanism of dynamic instability? Effect of MAPs on DI?

Goal: use computational modeling to **address these questions**, gain **intuitive feel** for MT systems

Computational models of MT dynamics

Requirements:

-Based on known structural, biochemical attributes

-Minimal number assumptions

Explicit and **intuitively understandable**

-Fast enough to allow simulation of a system of many MT over a biologically relevant span of time

Approach:

Borrow enthusiastically from existing work!

Chen and Hill PNAS 1985
Flyvbjerg et al., PhysRev 1994
Van Buren et al., PNAS 2002, BJ 2005
OTHERS!

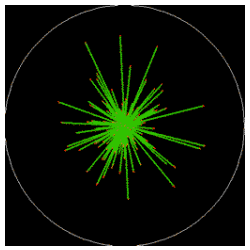
Adjust existing models to answer our questions

Two models, two scales, different applications

1) Mesoscopic or "microscope-scale" model

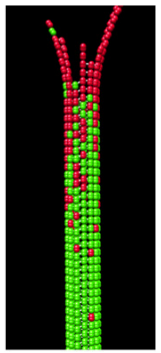
Influence of physical constraints on behavior of a system of MTs

Gregoretti et al. J Cell Sci. 2006



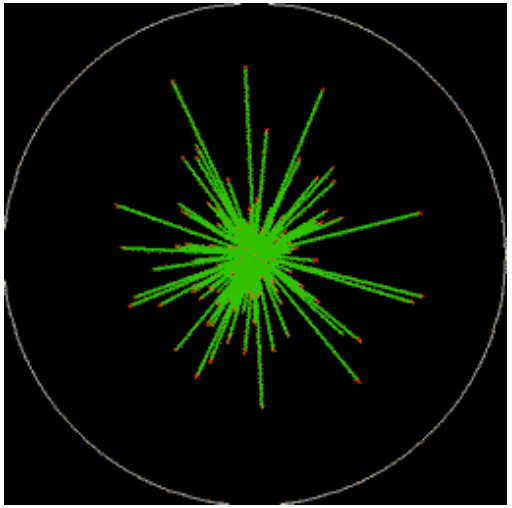
2) Microscopic or "molecular-scale" model

Mechanisms of catastrophe, rescue, MAPs
Effect of MAPs on systems of MTs



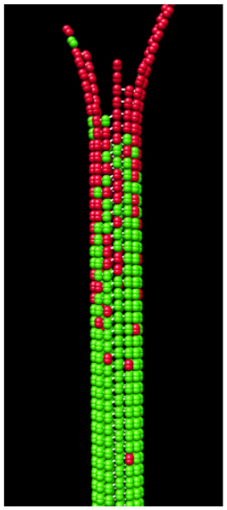
Both models: *relationship between behavior of* ***bulk polymer*** *and* ***single MTs***

Outline:
Biological Insights from Computational Modeling of Microtubule Dynamics



I. **Brief summary of what learned from study of mesoscopic model**

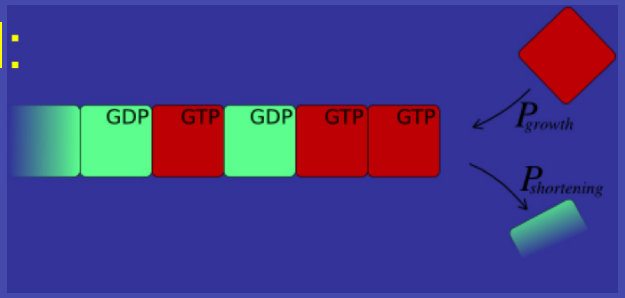
Gregoretti et al. J Cell Sci. 2006



II. Discuss findings with molecular scale model

- Mechanism of MT dynamics
- Revisiting concept of “critical concentration”

Key elements of "microscope-scale" model:



- "Monte Carlo" (stochastic) model
- MTs are simplified linear polymers

  obvious oversimplification...

- Polymerized tubulin has two states: "GTP" or "GDP"
  - states could represent other conformations

- Addition and loss of tubulin subunits, GTP hydrolysis occur according to defined probabilities

  - subunit addition depends on [$tubulin_{free}$]; loss does not
  - probabilities depend on the identity of the terminal subunit
  - GTP hydrolysis is stochastic, not vectorial

    ↪ Find parameters that give DI like that seen at interphase

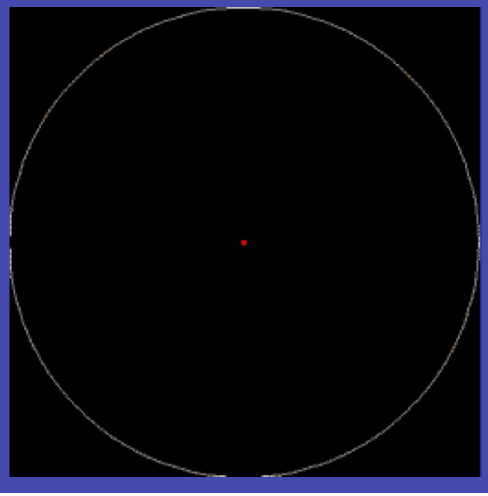
    Explore parameter space for robustness

User-defined parameters:
- [total tubulin]
- cell size
- # nucleation sites
- GTPase, k_{on}, k_{off}

Emergent parameters:
- growth, depol rates
- transition frequencies
- [polymer], [free dimer]

Differences:
- Model SYSTEM of competing MTs
  - MTs grow from defined numbers of nucleation sites
  - Growing MTs **compete** for unpolymerized dimers
  - DI parameters are **emergent, not assigned!**
- Addition of tubulin subunits can be limited by the cell "edge"

  (probability of tubulin addition is less likely)

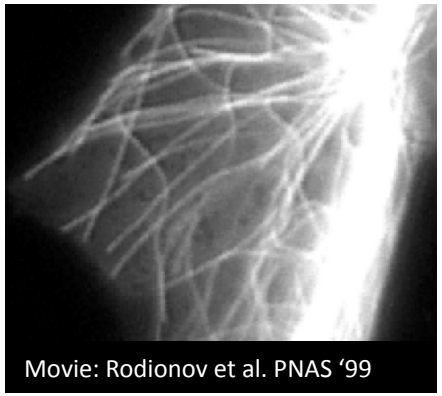


Questions for "microscope scale" model:

1) What causes persistent growth of MTs inside cells?

- growth in interior of cells is persistent
- classic dynamic instability out near cell edge

  Komarova et al., JCS 2002



Movie: Rodionov et al. PNAS '99

↪ Asymmetry in MT dynamics induced by interaction of MT with cell edge

Komarova et al., JCS 2002, Janson et al., JCB 2003

But: What causes persistent growth in first place?

MAPs? Gradient of MAPs??

2) What causes dramatic changes in MT dynamics through cell cycle?

interphase: MTs are long, persistent growth in interior

mitosis: MTs are short, dynamic

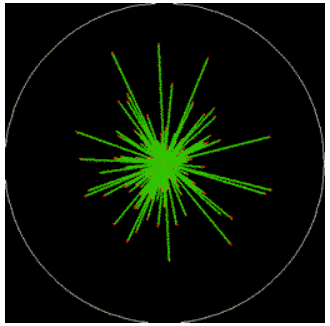
e.g., Rusan et al., MBOC 2001

↪ Regulation of MAPs induces changes

↪ Only answer??

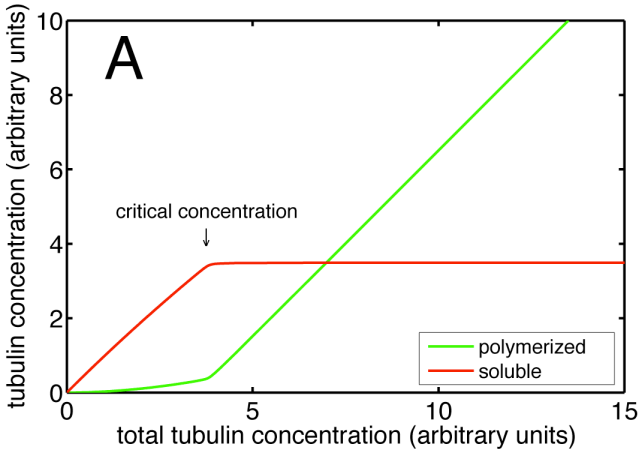
Biological conclusions from studying behavior of "microscope-scale" computational model

Gregoretti et al. J Cell Sci. 2006

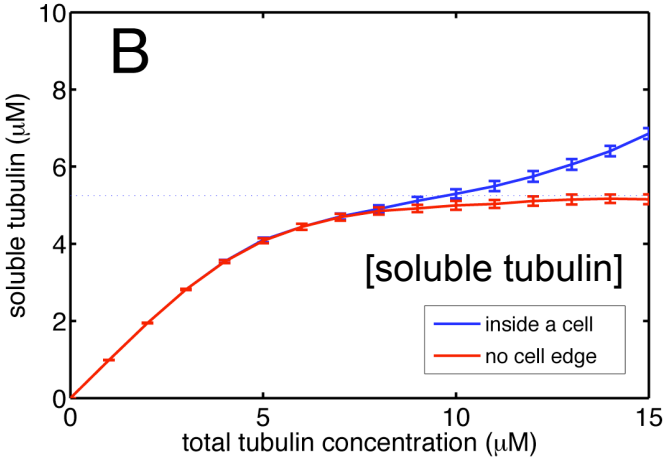


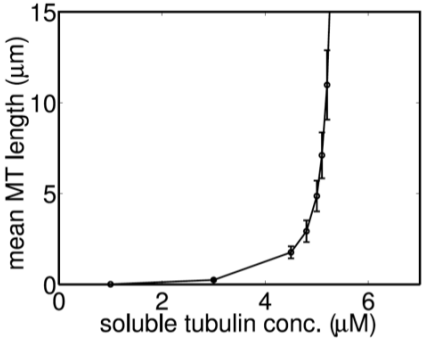
1. **Persistent MT growth** seen *in vivo* is an **unavoidable outcome** of putting sufficient tubulin in a confined space

Classical "critical concentration"



The model





Spatial limitation causes [free tubulin] to rise above the Cc

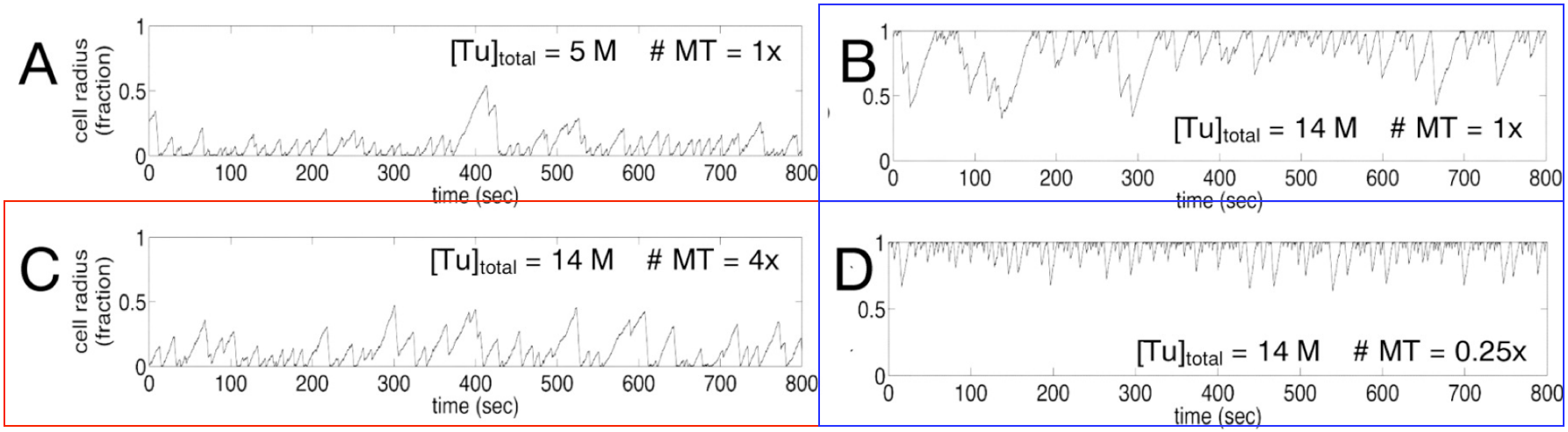
- more total tubulin → longer MTs
- long MTs reach cell edge
- cell edge induces catastrophe
- induction of catastrophe increases [free tubulin] above natural Cc

↪ Maps *involved*
Not *required*

Increased [free tubulin] ↑ rescue, ↓ catastrophe: *persistent growth*

2. Physical environment (cell size, # nuclei) influences MT dynamics

- Effects of physical constraint are **globa**l -- not just at cell edge
- Mutations that alter nucleation are *expected* to cause changes at + end
- Increase in MT dynamics seen at mitosis could be a simple outcome of the mitotic increase in MT nucleation



MAPs "tune" dynamics, but do not dictate them

- Even with MAPs, transition frequencies depend on [free tubulin]
  [free tubulin] depends on cell size, total tubulin, # nuclei…

  → *Cell simulations that fix DI parameters are missing part of the picture….*

Lots of remaining questions….

- **How do MAPs alter MT dynamics?**

  How would a lateral X-linker differ from a GTPase inhibitor?

- **How do MAPs alter systems of dynamic MTs?**

  Important to compare:

  - Confined systems (*in vivo*-like) - Non-confined systems (*in vitro*-like)

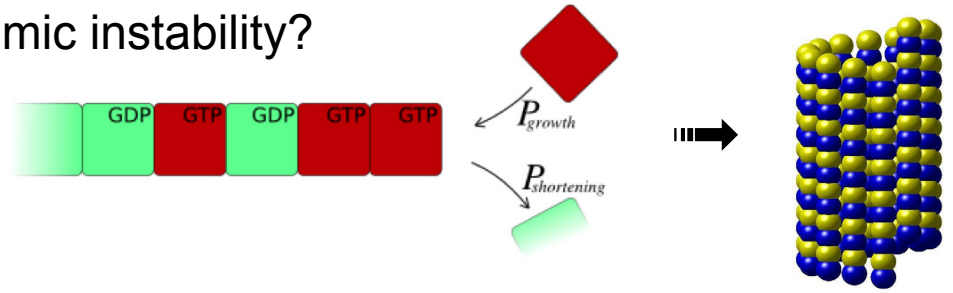
  ↳ *MAP effects won't always be the same!*

  ➙ Need for comparing in vitro expts to each other, in vivo work

- **How do MTs work?**

  What is the mechanism of dynamic instability?

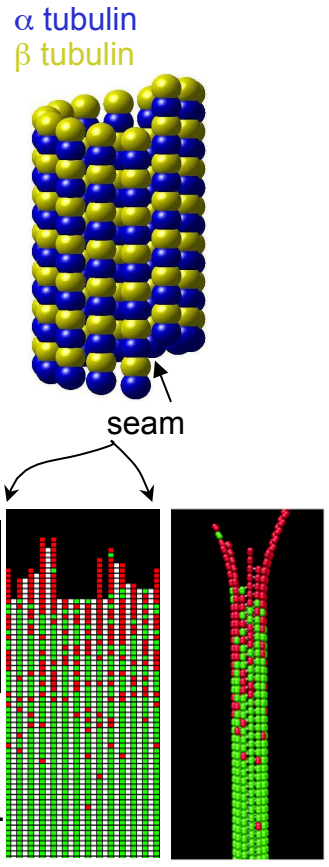
  Need more detailed model:



Additional Requirements for Molecular Scale Model:

- **Detailed** enough to incorporate MAP binding, release, varied activities
  - Account separately lateral and longitudinal bonds
  - Consistent with knowledge of **MT structure, biochemistry**
- **Fast** enough to allow simulation of individual MTs, systems of *many* MTs over tens of minutes (allow comparison to DI experiments)

Molecular Scale Model:



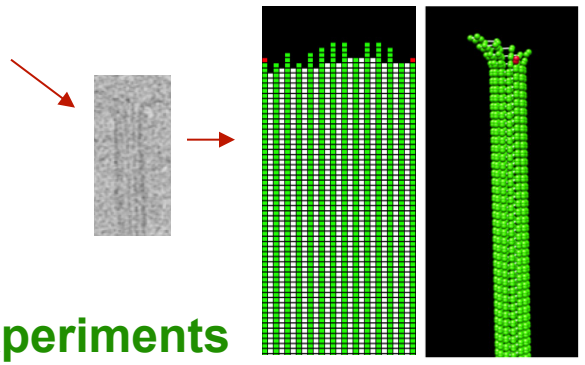
Approach:

- utilize existing work (VanBuren 2005)
- modify to optimize combination of structural detail and speed
  no need to reinvent wheel!

- **Similar to 'microscope-scale" model, but higher resolution**
  - cylindrical lattice of 13 protofilaments (13_3 lattice with seam)
    ↳ *projected as flat structure* *seam protofilament duplicated in visualization*
  - subunits: individual tubulin dimers
    - user defined values: rate constants, [tubulin], cell size…
    - emergent values: all else

Differences from previous models:

- **Interactions are consistent with structure** *not helical like Bayley's, Hill's….*
- **Lateral bonds are modeled *explicitly***
  - Bonds form, break according to user-defined rate constants
  ↳ laterally unbound GDP protofilaments ≈ "ram's horns"
    ↳ *visualized straight,* act *kinetically* as curved
    - exploration of the mechanisms of rescue, catastrophe
    - regulation by MAPs



All like VanBuren2005, but:

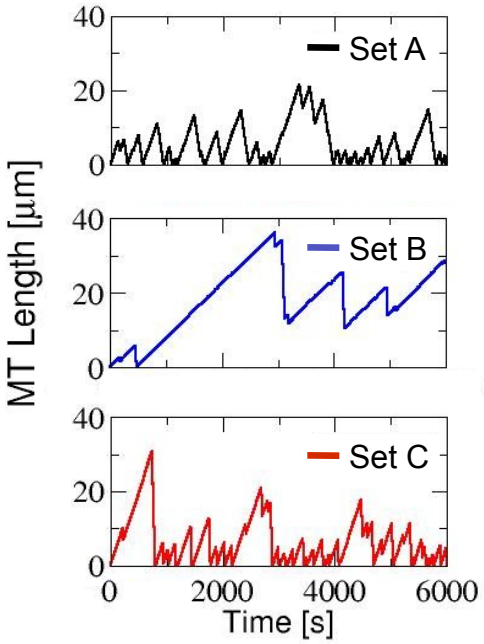
- **Simulate > tens of minutes: full dynamic instability experiments**
  - Mechanical influences on subunits approximated kinetically
    - presence of laterally bound neighbors influences lateral k_{bond}, k_{break}

First: Model recapitulates experimental MTs…

3 parameter sets: A,B,C

- Differ significantly
  e.g., $k_{h\text{-}A}$ = .2sec^{-1}; $k_{h\text{-}B}$ = .7sec^{-1}
- Focus on C: *most tuned to bovine-brain tubulin*
  Examine all three → parameter specific?

1. Displays dynamic instability (catastrophes AND rescues)



2. DI similar to BB tubulin

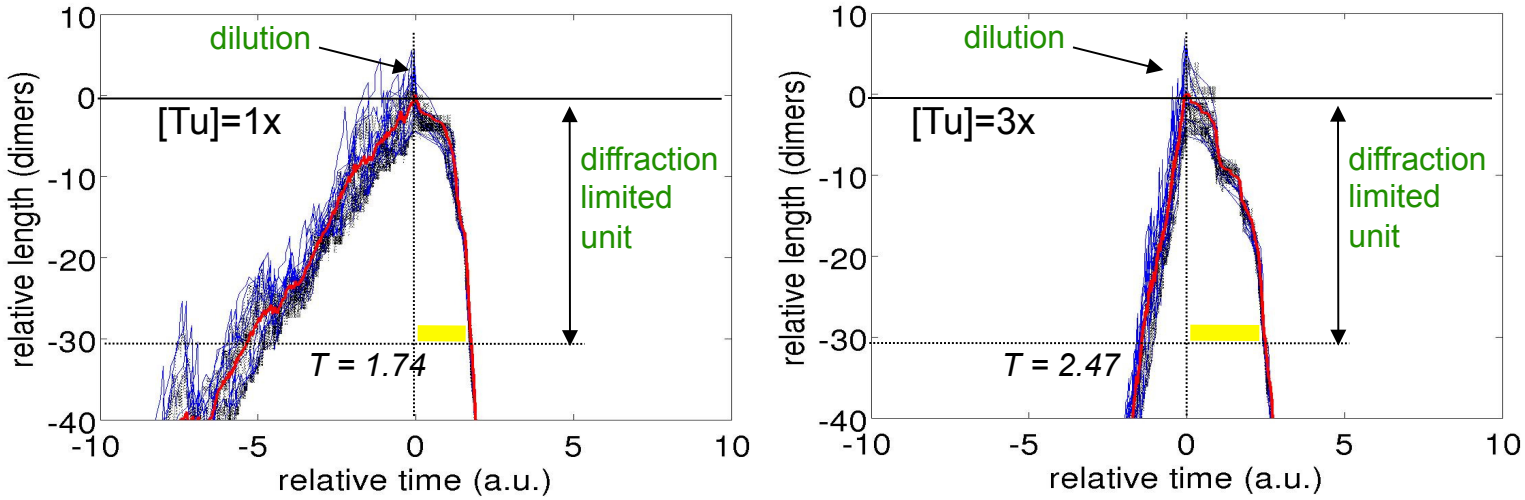
	Parameter Set	[tubulin] (μM)	K_h (aut^{-1})	V_g (dimer lengths/sec)	V_s (dimer lengths/ sec)	F_c	F_r	cap (dimer lengths)
Walker 1988 + others	A	14	0.2	5.31 ± 0.07	11.7 ± 0.1	0.00793 ± 0.0005	0.00243 ± 0.0007	25.3 ± 3.1
	B	10	0.25	1.84 ± 0.01	46.1 ± 4	0.00181 ± 0.0001	0.0405 ± 0.01	5.7 ± 1.2
	C	10	0.7	5.64 ± 0.04	60.8 ± 3	0.00962 ± 0.002	0.0185 ± 0.007	8.8 ± 1.5
Experiment:		10	0.2-1	~3	~60	0.008	0.024	1-20

3. Reproduces experimental "sudden dilution" experiments:

➤ pre-dilution growth velocity has little effect on "time to catastrophe"

Parameter Set	Time to depolymerization (s)	
	10 μM [Tu]	30 μM [Tu]
A	4.44 +- .57	4.29 +- .78
B	2.23 +- .44	3.39 +- .39
C	1.78 +- .47	2.5 +- .30

↪ has been used to argue against existence of extended GTP cap

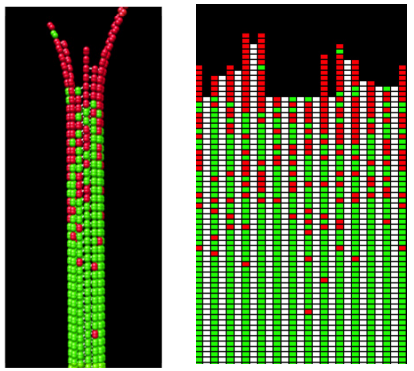


Summary: all as expected

In addition:

4. **GMPCPP**-bound MTs are **stable to dilution** over extended time (set C)
5. **Dependence** of DI parameters on **[tubulin]** does show some **deviation**…

Inferences/Predictions from molecular-scale model:



Shape of tip during growth:

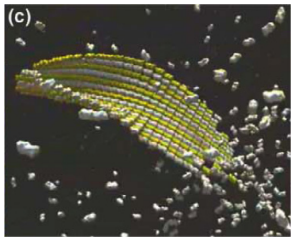
1. Closed tube with multi-protofilament extensions

regardless of parameter set

➛ **Not able to observe expected open tube**

Considerable tuning: *no open sheets*

weaker seams: more frequent catastrophes



Nogales COCB 2006

↪ **Failure to observe sheets due to failure of model?**

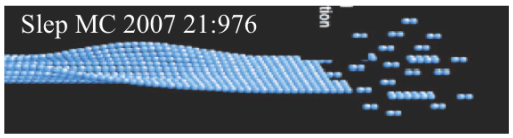
Suggest: Reconsideration of idea that MTs grow as sheets

a) CryoEM work showing “sheets” seems equally consistent with extensions

Chretien, 1995

b) Growth of an extended open sheet implies lateral bonds > longitudinal bonds

- Otherwise subunits at sheet edge should fall off resolve sheet to tube
- Blunt sheets particularly unlikely



2. Cracks between protofilaments exist even in growing tips

Expected from:

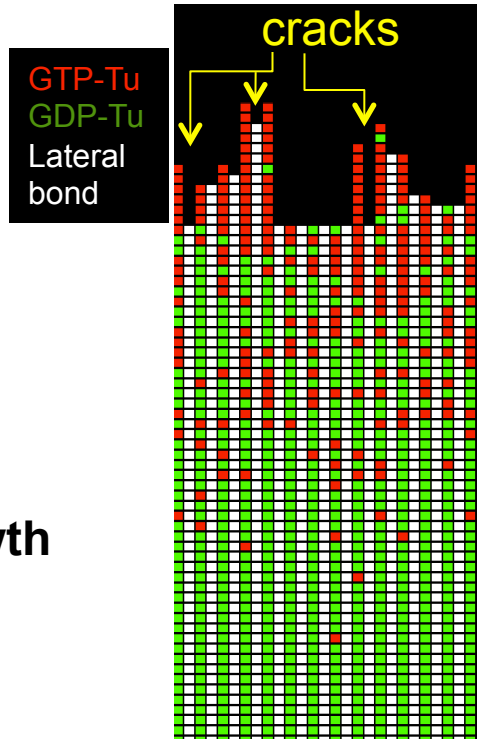
- Entropic considerations

  unlikely that all bonds form simultaneously

- Longitudinal bonds stronger than lateral

  (VanBuren 2002, Sept 2003)

∴ laterally unbonded regions at tip



Consequences:

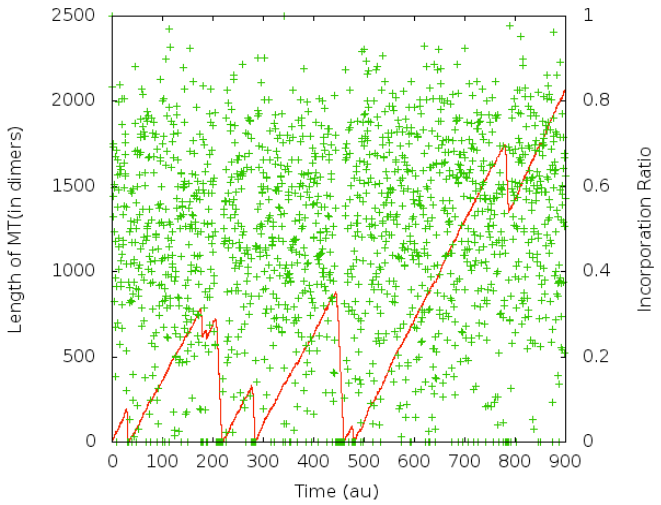
- **Large fraction** of of attached subunits **detach during growth**

  (Walker et al., 1991, Scheck et al., 2008, Odde this meeting)

- ***Any* subunit likely to detach before laterally bonded**

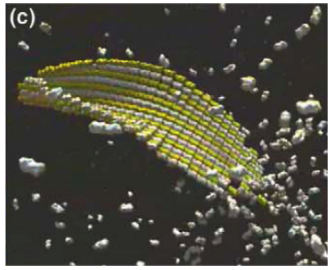
  not just those in "unfavorable" environments

Fraction of subunits incorporated sec^{-1} (Set C)



3. Simple 1st order GTP hydrolysis on non-terminal subunits is sufficient to account for dynamic instability

- no need for vectorial hydrolysis
- no need for sheet-closure → to explain catastrophe



Nogales COCB 2006

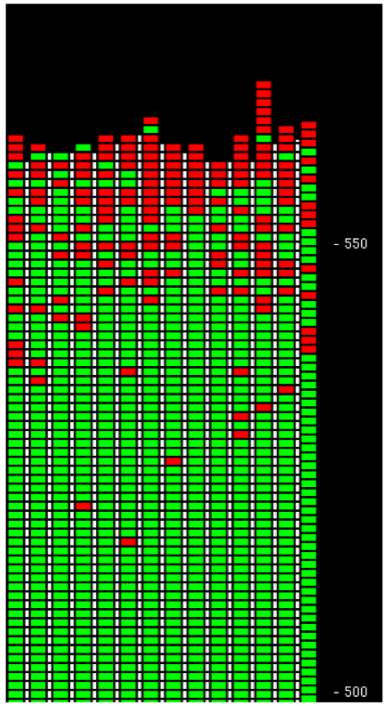
4. GTP cap is:

- ***not* a well-defined structure with discrete edges**
- a heterogeneous, dynamic, functionally defined entity
  - region rich in laterally bonded GTP dimers?
  - *effective* cap < total # GTP-Tu
- too short, short-lived to detect by most methods
  - why so hard to detect cap

Predicted by other models (VanBuren 2005)

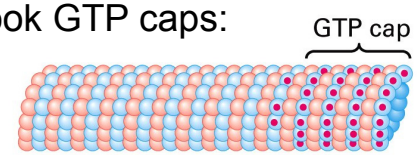
Consistent with experimental data

But: idea of solid cap, vectorial hydrolysis persist

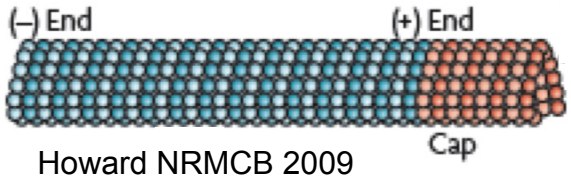


Dilution simulation
Set C
~real time

Textbook GTP caps:



Lodish MBOC 2004



Howard NRMCB 2009

5. GTP hydrolysis reduces strength of longitudinal bonds

Generally assumed: GDP weakens only lateral bonds

Evidence:

Required to match behaviors of GTP *and* GMPCPP MTs

GMPCPP: slowly hydrolyzable GTP analog

GMPCPP MTs: extremely stable

↪ GMPCPP MTs: strong constraint for GTP-Tu state!

Sets A, B: life-like DI with GTP-Tu

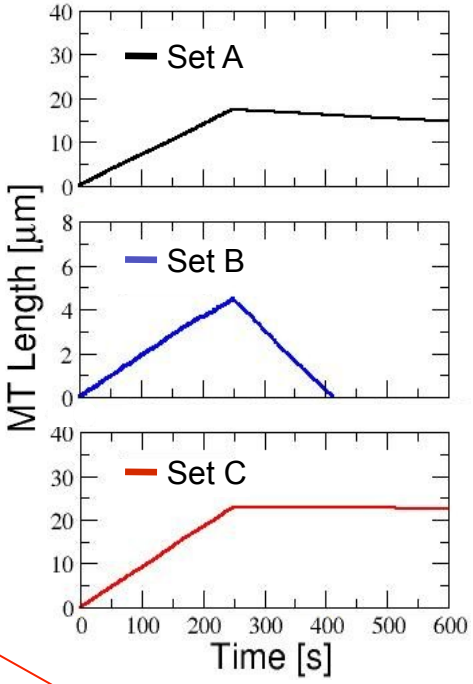
➢ **GMPCPP-Tu: Sets A, B depolymerized *too quickly***

- Could not identify set that matched GTP and GMPCPP *unless* changed model
- GTP/GDP affects lateral *and* longitudinal bonds - **Set C**

Consistent with nearness of nucleotide to dimer interface, structural changes at interface:

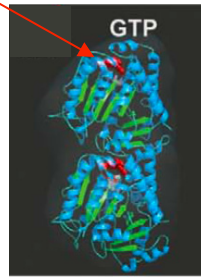
Rice PNAS 2008; Nogales, COCB 2006

Depolymerization of GMPCPP MTs after removal of soluble tubulin



Unique (?) among computational models of MT dynamics

➢ importance of tuning parameters to many types of experimental data



Nogales COCB 2006

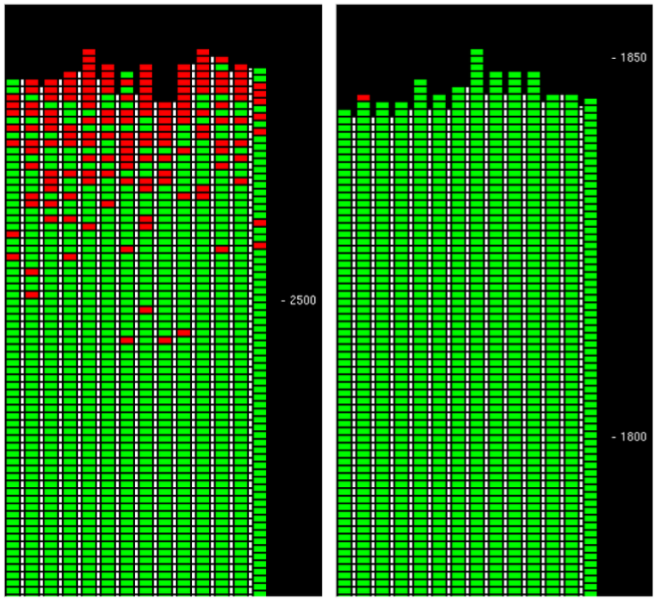
Mechanisms of rescue and catastrophe??

Observation:
- Growing MT likely to keep growing
- Depolymerizing MT likely keep depolymerizing

➥ **What "tips the tip" to transition?**

Expect:
Some obvious attribute of tip structure will predict transition:

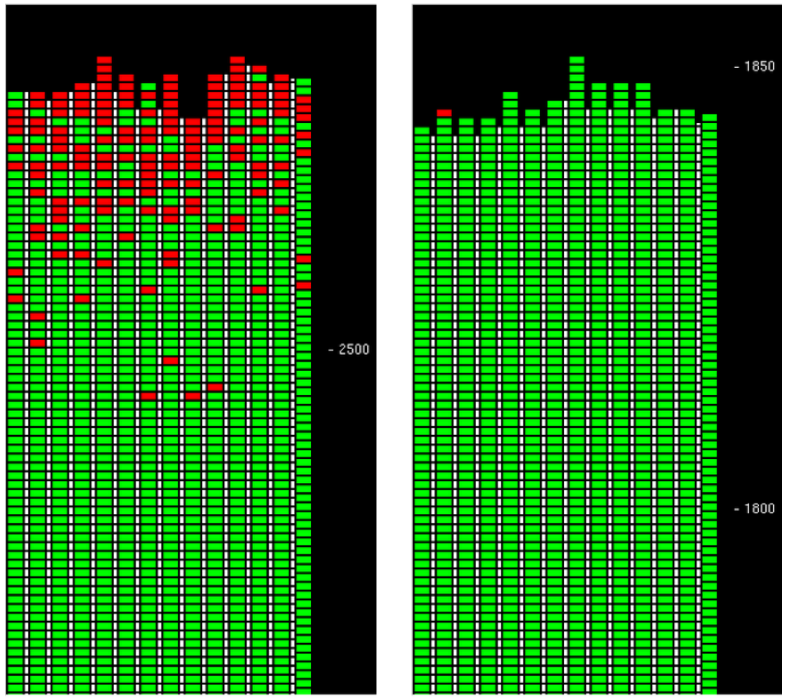
- number of GTPs in cap
- tip "raggedness"
- depth of inter-protofilament cracks
- length of laterally bonded GTP cap



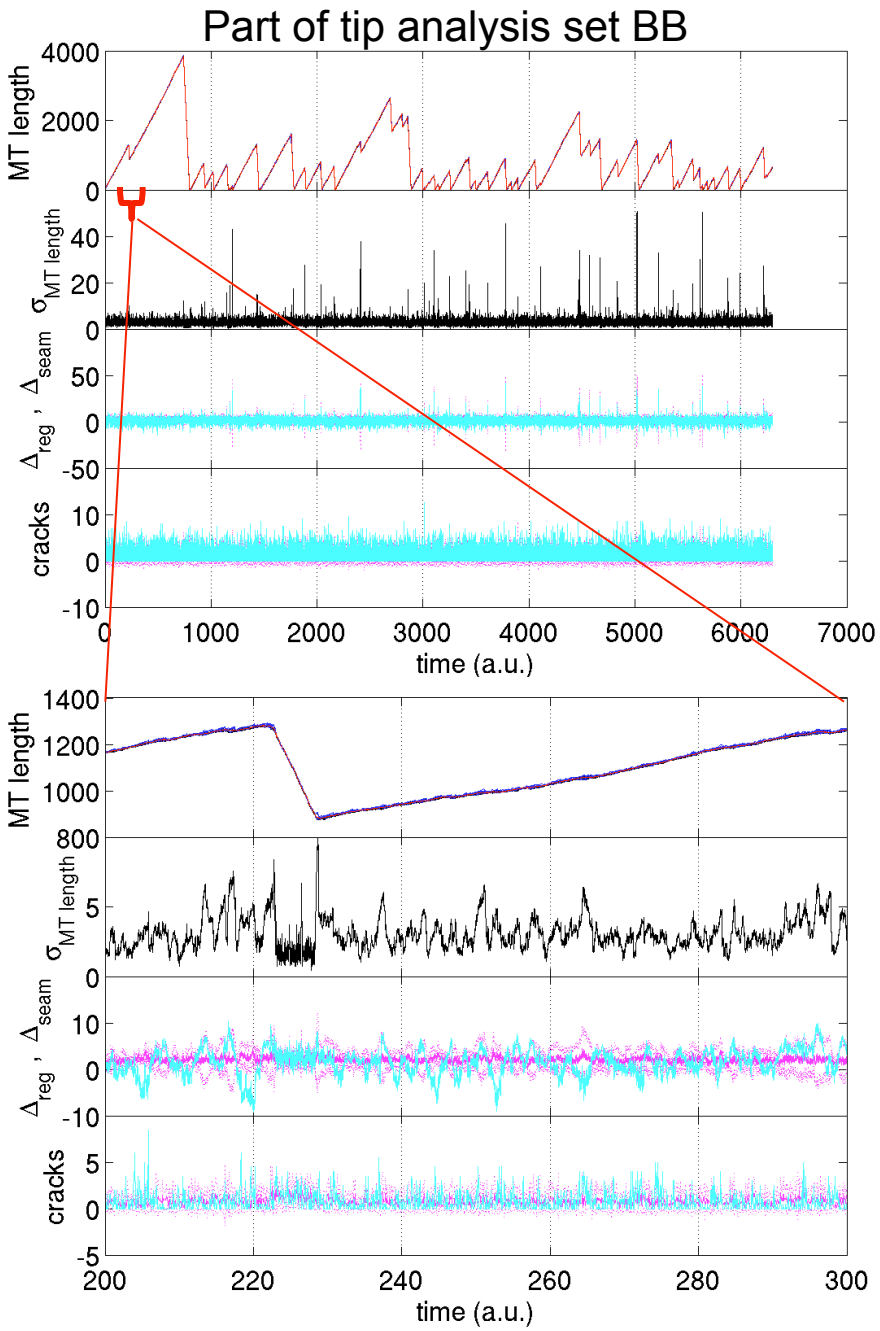
Observation:

- No one attribute "predicts" catastrophe or rescue

  ↳ Tip fluctuates too quickly!

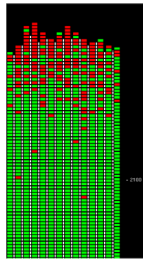


6 frame per sec ~real time



Another Approach

- Identify, characterize true "tipping point" MT tip structures
  - take "snapshots" of tip configurations during transitions
  - use these configurations as starting point for 10 new simulations
  - find, study subset of structures that can "go either way"

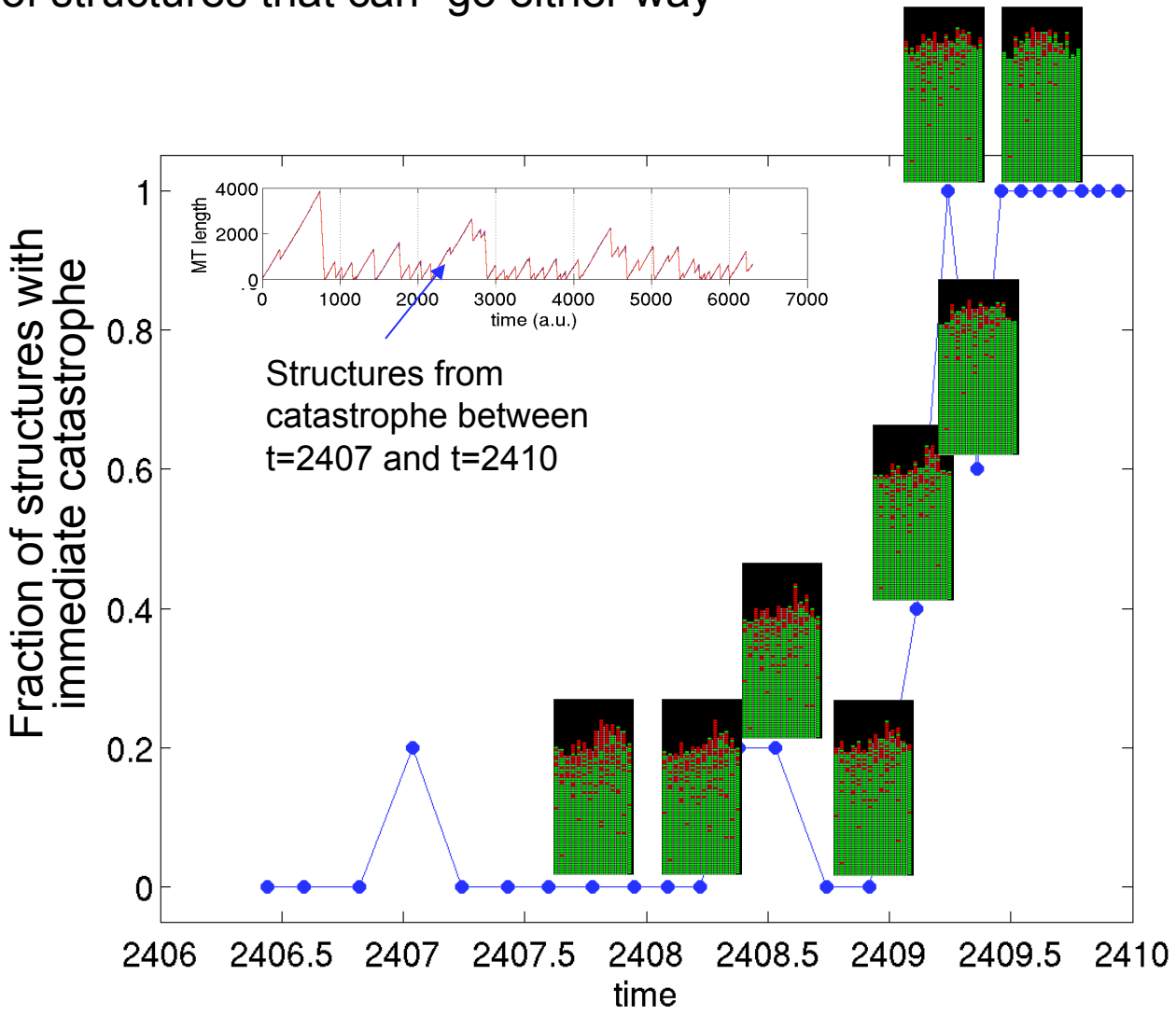


"Tip fate analysis"

Transition likelihood for naturally occurring tip structures

shown as images

5 simulations each structure

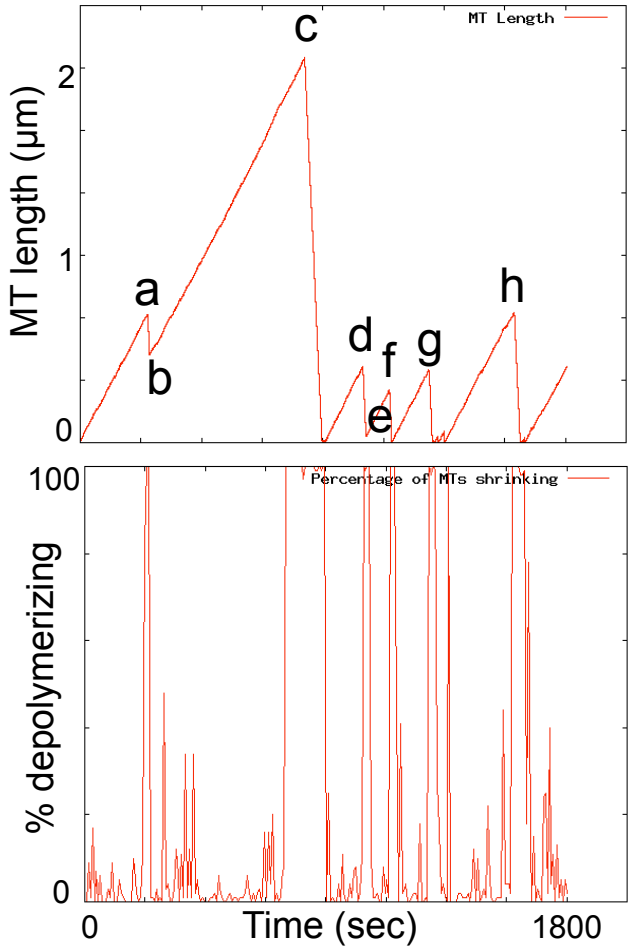


Conclusions:

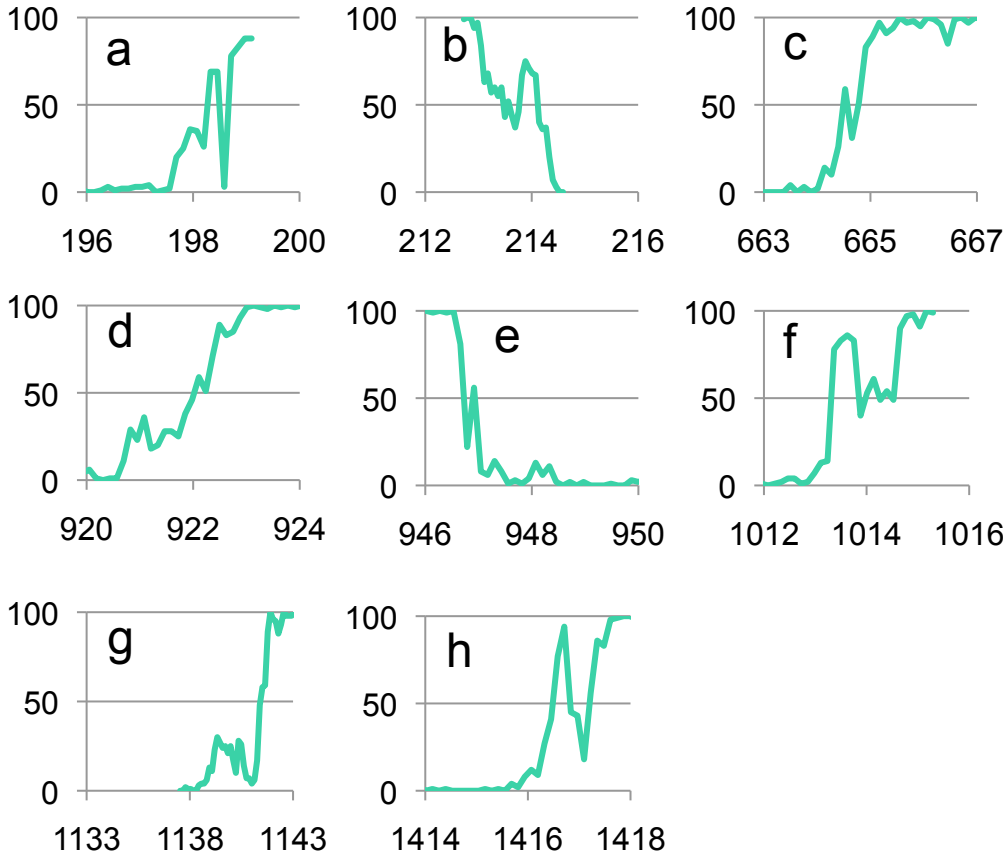
- **True transition-prone structures are rare**
- **Transition is *fast* (1-2 sec)**

Test predictions:

- Sample structures from full life-history plot
  - 1 sample/sec
  - 100 new simulations/tip structure



- Examine transitions more systematically
  - 10 samples/sec
  - 100 new simulations/tip structure



True transition-prone structures *are* rare
Transitions happen in 1-2 seconds

Features of transitional structures?

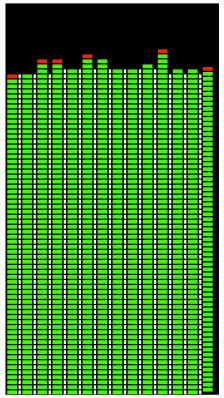
→ No universal attribute

Not simply more/less GTP

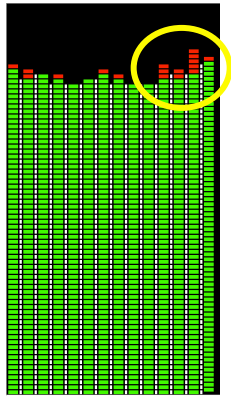
Rescue:
Becomes likely when have a few *laterally bonded* GTPs…

Catastrophe:
Correlates with cracks extending into GDP-rich region…

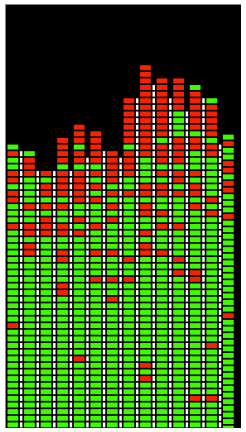
Depol ~100%



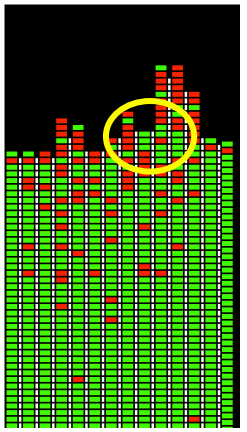
Rescue ~50%



Grow ~100%



Catastrophe ~50%



Propose:

➢ Fluctuations in depth, distribution of **cracks** play **pivotal role in DI**

↪ provides mechanism for action of MT binding proteins

Conclude

➢ **MT dynamics best explained by refinement of "fluctuating cap model" originally proposed by Chen and Hill (1984)**

Stochastic cap model:

- **MTs hydrolyze GTP according to 1st order rate constant**
- **Catastrophe and rescue result from stochastic fluctuations in shape, depth, and lateral bonding of the cap**

In addition: **MTs grow as closed tubes with extensions**

Why support this conceptual model?

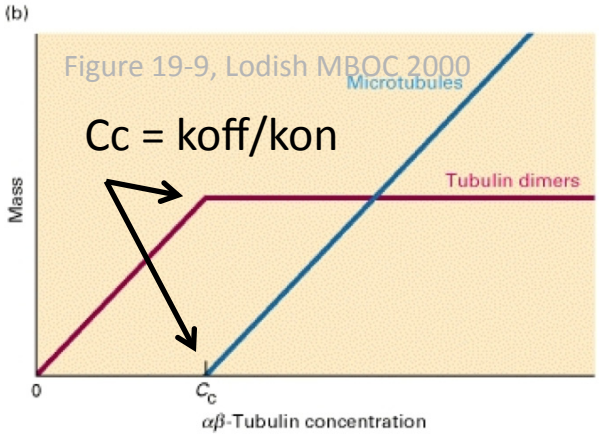
not simply that it produces life-like dynamic instability

➢ *Lots* of models, multiple real systems produce dynamic instability

Question to ponder:

➢ **What characteristics of a system are necessary to produce DI?**

Re-evalulation of "Critical concentration"



➥ **Why??**

Commonly used concept:

- ~150 papers on MTs have "critical concentration" in *abstract*
- Discussed in cell biology textbooks: Pollard, Lodish, Alberts…
- Cell biologists/biochemists use Cc concept to design and interpret experiments

What IS the critical concentration?

3 textbook definitions:

1) Concentration of subunits needed to get polymer assembly
2) Concentration of subunits in solution once steady-state is reached
3) Equilibrium constant for binding of monomer to polymer

Problem: relationships above were derived for *equilibrium polymers*

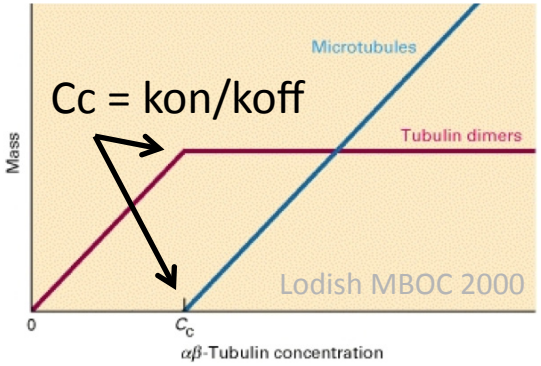
MTs and actin are *steady-state polymers*: energy is used in polymerization process

➤ **How should understanding of critical concentration be modified for steady-state polymers?**

To begin, look back to textbook definitions:

1) Concentration of subunits needed to get polymer assembly "Cc_1"
2) Concentration of subunits in solution once steady-state is reached "Cc_2"
3) Equilibrium constant for binding of monomer to polymer Cc kon/koff "Cc_3"

All equivalent:
$Cc_1 = Cc_2 = Cc_3$
"THE critical concentration"



Questions:

- Are these definitions still **valid** when considering **steady-state polymers?**
- Are valid definitions still ***equivalent?***
  - ➥ **One** critical concentration, or **more?**
- How do these critical concentrations **relate to dynamic instability behavior?**
- How do they relate to **biochemical rate constants?**

Goal: Develop a more complete and **intuitive understanding** of

- behavior of populations of biologically relevant polymers
- How this population-level behavior relates to behavior of individual filaments.

Focus: Microtubules

Approach: computational models of MT dynamics

1. Are these definitions still valid when considering steady-state polymers?

Start with the easy part:

3) Equilibrium constant for binding of monomer to polymer: Cc = koff/kon

Well-established that definition #3 is not valid:

- GTP and GDP forms of tubulin have different k_{on} and k_{off} values
  Different Cc values for GTP and GDP tubulin

-> "Real" Cc will be in between Cc_GTP and Cc_GDP

Cc_GMPCPP < 1µM
Cc_GDP ….> 20µM??
1µM < Cc < 20µM

Sophisticated textbooks (Alberts) note this
others (Lodish) don't

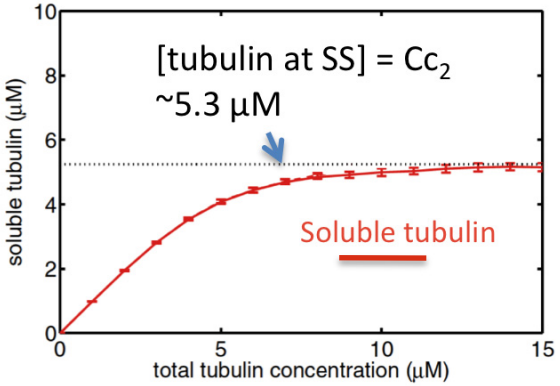
2. Are remaining definitions of Cc equivalent in steady-state polymer systems??

1) Concentration of subunits needed to get polymer assembly "Cc_1"
2) Concentration of subunits in solution once steady-state is reached "Cc_2"

↪**Hint that they are *not* equivalent form work with "microscope-scale" models**

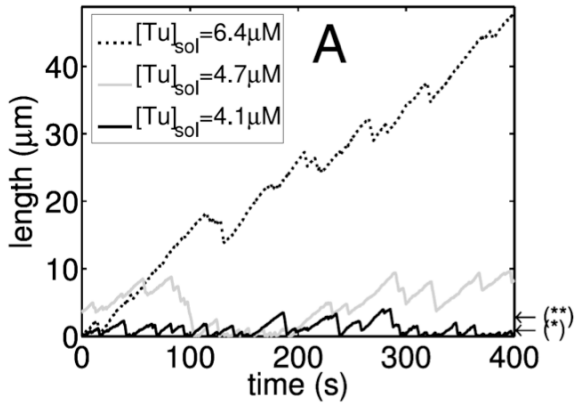
Gregoretti JCS 2006

Examine behavior of population of dynamic MTs



Prediction: No MTs below Cc_2

Examine individual MTs

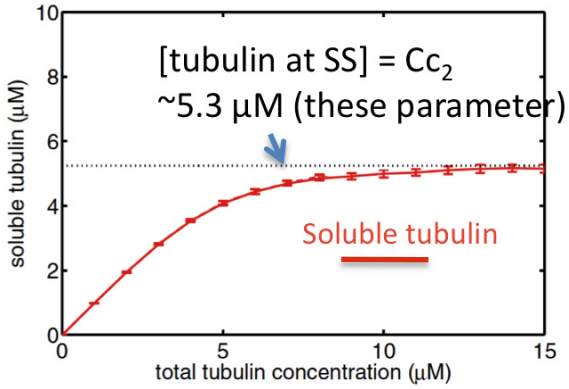


Observation: MTs are growing at [tubulin] *lower* than Cc_2! (same parameters)

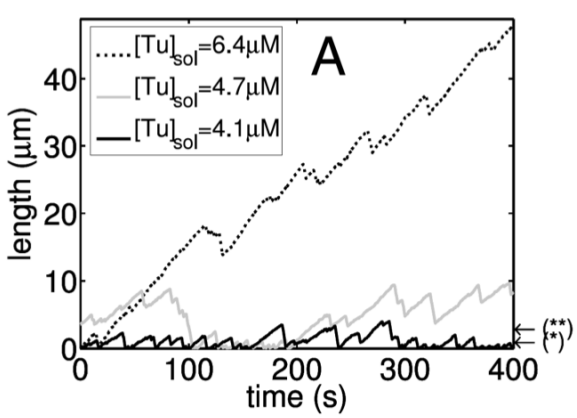
➔ **Suggests two definitions of Cc are *not* equivalent: $Cc_1 \neq Cc_2$**

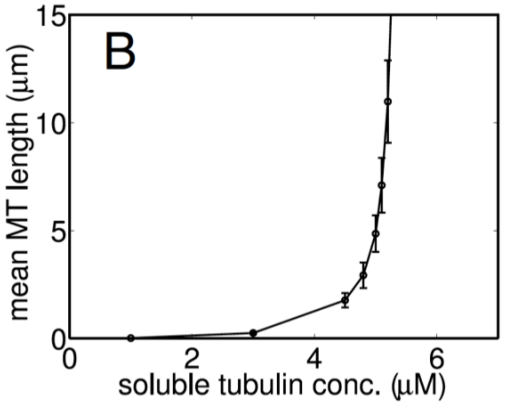
➤ If Cc_2=[$tubulin_{SS}$] is ***not***
"the concentration of subunits needed for MT growth"

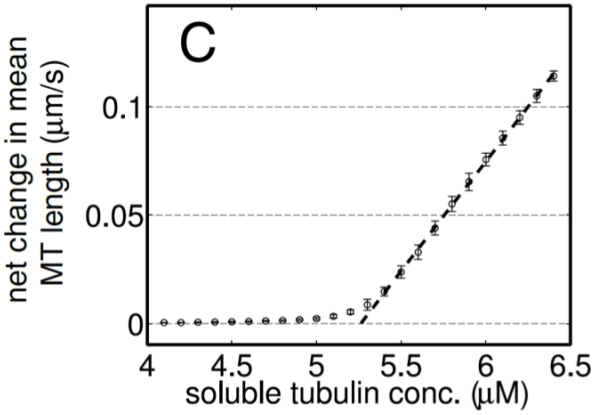
→ What IS Cc_2 ?



Examine behavior of MTs in steady-state systems in more detail







➢ **[$tubulin_{SS}$]= Cc_2 is the concentration of tubulin needed for *persistent growth***
Also called "unbounded" growth (e.g. Verde et al. 1992)

New (?) definition for [free tubulin] in pool of dynamic MTs at steady state:
[$tubulin_{SS}$] = Cc for persistent growth -> "Cc_p"

Note: Cc_p is the *asymptote* that is approached: [$tubulin_{ss}$] is not actually a constant

Conclusions of CC work *in progress*

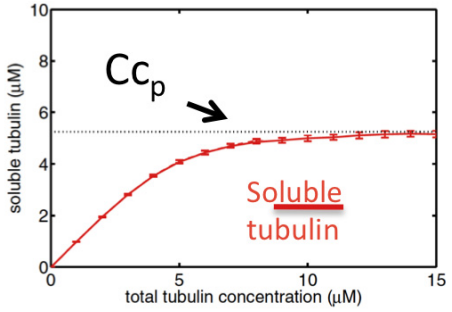
At least two different "critical concentrations"

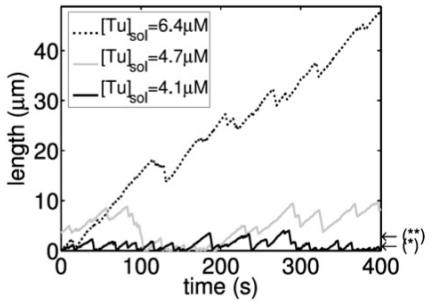
$Cc_2 = Cc_p = [tubulin_{SS}]$= [tubulin] needed for persistent growth

persistent growth = unbounded growth

~10µM experimentally in vitro

~12 µM detailed model standard parameters





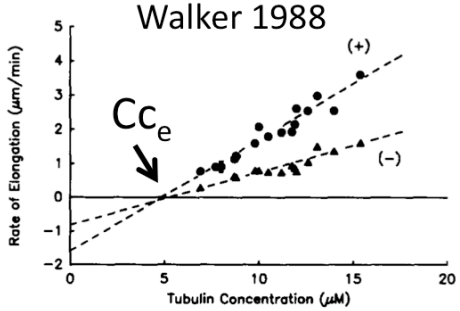
$Cc_1 = Cc_e$ = [tubulin] needed for MT elongation

k_{on}[tubulin] > k_{off}

~3-5µM experimentally

~12 µMdetailed model standard parameters

Walker 1988

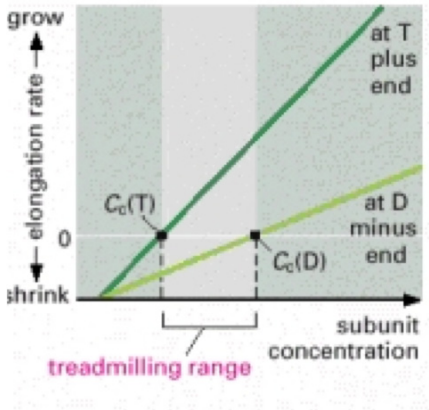


"Regular" MT dynamic instability occurs *between* these two concentrations

Treadmilling??

Should occur when [tubulin] > Cc_p for +end?

> Cc_e is not sufficient!



Alberts textbook:
(also Howard)
-> Not correct!